Biochimica et Biophysica Acta, 644 (1981) 273–283
Elsevier/North-Holland Biomedical Press

BBA 79273

DRUG-INDUCED ZETA POTENTIAL CHANGES IN LIPOSOMES STUDIED BY LASER DOPPLER SPECTROSCOPY

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(Received November 26th, 1980)

Key words: Laser Doppler spectroscopy; Surface charge; Liposome; β-Blocker; Anesthetic; pH dependence

The technique of laser Doppler spectroscopy is used to measure the electrophoretic mobility of liposomes under the influence of one β-blocking agent and three local anesthetics. All four drugs decrease the mobility (i.e., the zeta potential) of negatively charged phospholipids (soybean lipids, phosphatidylserine and cardiolipin). The mobility of electrostatically neutral pure phosphatidylcholine (zero mobility under control conditions at pH 7 and 4) is increased linearly with the logarithm of drug concentration, indicating binding and incorporation of positively charged drug molecules. The sequence of strength of activity, measured by zeta-potential changes, corresponds to that found in biological tissues: propranolol > tetracaine > lidocaine > procaine. For purely negatively charged lipids (phosphatidylserine, cardiolipin) the activity of the drug is higher at acidic pH, (pH 4), while for electrostatically neutral (phosphatidylcholine) or partly neutral (soybean) lipid liposomes drug activity is about the same at pH 9, 7 and 4. A Hill plot of the data reveals noncooperative drug binding. From the line width of the scattering power spectrum the mean particle radius and the average interparticle distance in the samples are determined.

Introduction

Most of the membranes which occur in biological tissues are composed of charged lipids and proteins. Fixed electrostatic charges will produce an electric potential between the membrane surface and the bulk ionic atmosphere. This potential is one component of the total membrane potential in biological tissues and is, in principle, measurable by electrophoresis, in which charged particles move in an applied electric field according to their charge density. The fundamental theory and experimental procedures are extensively described in books by Shaw [1] and Aveyard and Haydon [2].

The surface potential plays an important role in the interaction of charged drugs with biological membranes. Besides ionic interactions, dipole and Van der Waals' forces may also contribute to the binding of a molecule.

When a charged molecule is bound to a membrane surface, the surface potential is changed. Such a change could have important consequences for the functions of the membranes. As shown by Gilbert and Ehrenstein [3] and by Hille et al. [4], positively charged divalent ions strongly adsorb to negatively charged acidic groups. The field-dependent sodium and potassium channels may be influenced by these local field changes. As a result, the conductance-voltage curves of axons would be shifted to higher voltages. The divalent ions may exert their effects by screening as well as by binding to the negative sites [5,6]. Many investigators have presented extensive work on the binding of Ca^{2+} to phospholipid monolayers [7–9], lipid vesicles [8,10–15] and bilayer membranes [6,16]. The interaction of local anesthetics with artificial lipid structures has also been studied [10,16–19] with regard to changes in surface potential. As proposed by McLaughlin this

interaction is hydrophobic in nature [17]. Little is known about the interaction of β-adrenoceptor blocking agents with phospholipids [18,20]. This type of drug, which specifically interacts with β-receptors, also develops an antiarrhythmic- or local anesthetic-like effect on the heart.

The commonly used electrophoretic method for the investigation of surface charge can only be applied to relatively large particles, the movement of which can be observed under the microscope.

A new technique has been developed in recent years by which particle mobility is measured by Doppler shifts of laser light [21–23]. This laser Doppler electrophoresis has been successfully applied for the characterisation of the surface groups of blood cells [24,25], chromaffin granules [26], synaptic vesicles [27] and mast cells [28].

A slightly modified technique of quasi-elastic light scattering in the absence of an electric field has been applied by Selser et al. [29] for the biophysical characterisation of sarcoplasmic reticulum vesicles. This method is suited to measuring the size and estimating the size distribution of bioparticles.

For comparative studies, the effect of three local anesthetics of different potency and one β-receptor blocking agent on the surface potential of phospholipid vesicles of different lipid composition has been investigated by laser Doppler electrophoresis. Furthermore, particle size has been measured by quasi-elastic light scattering.

Materials and Methods

Phospholipids. Phosphatidyl-L-serine from bovine brain in a chloroform/methanol solution (Koch-Light-Laboratories), cardiolipid as a sodium salt from bovine heart in an ethanol solution (Sigma), and crude L-α-phosphatidylcholine Type II-S from soybeans were used as received. As shown by thin-layer chromatography, phosphatidyl-L-serine and cardiolipin gave one single spot, whereas soybean lecithin contained, besides phosphatidylcholine and phosphatidylethanolamine, phosphatidylserine and cardiolipin. Pure egg yolk phosphatidylcholine in chloroform was kindly donated by L. Michaelis, Department of Biochemistry, University of Hull, U.K.

Drugs. Drugs were kindly provided by manufacturers: tetracaine- and procaine-hydrochloride (Hoechst), lidocaine-hydrochloride (Pharma-Stern), propranolol-hydrochloride (ICI-Pharma).

Preparation of lipid vesicles. Phosphatidylserine, cardiolipin or purified phosphatidylcholine solutions were used at a concentration of 1 mg/ml. The organic solvent was evaporated under vacuum and then purified N_2 was passed over the dried phospholipid. A buffer solution was added, containing KCl and glycine in a concentration of 1 mM each and adjusted to pH 8.0 with KOH. The suspension was hand shaken for 5 min befor it was sonicated for 30 min in a probe-type sonicator (Braun Sonic 125, tip diameter 0.5 inch) at a nominal frequency of 20 kHz and medium power level; the hand shaken cardiolipin liposomes were used directly.

Soybean liposomes were prepared by adding buffer to the solid substance to give a concentration of 1 mg/ml. The suspension was stirred overnight with a magnetic stirrer. Electrophoretic mobility of cardiolipin and soybean liposomes was not influenced by sonication. Only the linewidth of the laser power spectrum increased with increasing degree of sonication, which made accurate reading of the frequency more difficult. All liposome suspensions were diluted 1/3 (v/v) with the same buffer solution before the experiment was performed.

It is clear from the size measurement that there is a high percentage of multilamellar liposomes. This is of no importance for the drug effects as electrophoresis 'sees' only the surface of the particle and not its interior.

Experimental procedure

The instrumental arrangement for measurements of electrophoretic mobilities by laser Doppler spectroscopy has been described before [23]. The electrophoresis cell was made of plexiglas and consisted of four compartments in series. Platinum electrodes were fixed to the outer buffer compartments to apply constant voltages. The two inner sample compartments were separated from the electrode compartments by dialysis paper of small pore size to prevent the diffusion of liposomes and drugs into the electrode chambers. The sample compartments, with a total capacity of 5 ml, were connected by a glass capillary bridge coated with methylcellulose. For a given measurement, the same buffer solution was used for all the compartments.

Buffer solutions of various concentrations and pH were utilized. Concentrations were not allowed to be higher than 11 mM in order to avoid Joule heating phenomena. All experiments were performed at room temperature (20–23°C).

A constant potential of 100 V was applied to the cell, unless otherwise stated. In order to minimize polarisation effects on the electrodes, the polarity was automatically switched every 15 s. One measurement lasted 64 s.

Under the voltage, liposomes started to migrate across the capillary, on which the beam of the helium-neon laser was focused. The scattering light was detected with a photomultiplier and analyzed after preamplification in a spectrum analyzer. The spectrum was monitored on an oscilloscope with which the Doppler shift in frequency could be measured directly. A single-longitudinal He-Ne laser (Spectraphysics) of 15 mW strength and 6328 Å wavelength was used in all experiments.

The instrumental deviation was less than ±0.5%. The mobilities of different liposome preparations varied by ±2.5%.

Equations. The electrophoretic mobility was calculated from the Doppler shift in frequency $\Delta\omega$ according to

$$\Delta\omega = \mu(E \cdot K) \tag{1}$$

where μ is the electrophoretic mobility (μm/s per V per cm) and E the applied electric field (V/cm). The scattering vector K is determined by

$$K = \frac{4\pi}{\lambda} \sin\frac{\vartheta}{2} \tag{2}$$

where λ is the wavelength of the helium-neon laser, ϑ is the angle at which the experiment was performed, i.e., the angle between the incident laser light and the photomultiplier. Unless otherwise stated, experiments were performed at $\vartheta = 15°$. Mobilities were converted to ζ-potentials by Henry's equation:

$$\zeta = \frac{3\eta\mu}{2\epsilon_r\epsilon_0 f(\kappa a)} \tag{3}$$

where η is the viscosity, ϵ_r and ϵ_0 are the relative and absolute dielectric constants and $f(\kappa a)$ is Henry's function, which varies between 1.0 and 1.5 as (κa) goes from zero to infinity; a is the particle radius and $1/\kappa$ is the thickness of the diffuse double layer, which is calculated by

$$\kappa = 0.328 \cdot 10^8 \sqrt{cz^2} \tag{4}$$

where c is the electrolyte concentration and z the valency of the symmetrical electrolyte.

The charge density σ at the surface of shear is calculated from

$$\sigma = \frac{\sqrt{c}}{136.6} \sinh\frac{F}{2RT}\,\zeta \tag{5}$$

where σ is in $e^-/\text{Å}^2$, R gas constant, F Faraday constant and T the absolute temperature.

Binding constants are determined according to

$$K_a = \frac{1}{\sigma}(\sigma_{\text{initial}} - \sigma)\,C_0 \tag{6}$$

where σ_{initial} and σ are the charge densities before and after application of the drug and C_0 is the drug concentration at the plane of shear. C_0 is related to the bulk concentration c by the Boltzmann expression

$$C_0 = c \cdot e^{-(F/RT)\zeta} \tag{7}$$

The mean effective dose (ED_{50}) is defined as the concentration required for half-maximal drug effect. When the Boltzman law of drug distribution between bulk phase and receptor is not taken into account, K, the dissociation constant for the drug-receptor complex, can be set equal to ED_{50}. In our experiments we calculated ED_{50} by fitting the logit function to the experimental data using the least-squares method [30]:

$$y(x) = \frac{100}{1 + 10^{-s(x - \log ED_{50})}} \tag{8}$$

where s = slope and x = log concentration.

From the quasi-elastic light scattering experiments in the absence of an electric field the mean particle size is calculated from

$$\Gamma = K^2 D \tag{9}$$

where Γ is the half-width of the frequency power

spectrum and D the diffusion coefficient.

$$D = kT/6\pi\eta a \qquad (10)$$

where k is the Boltzmann constant, T the absolute temprature, η the viscosity and a the particle radius. The power spectrum was subjected to Fourier transformation to yield the autocorrelation function.

Results

Typical Doppler spectra are shown in Fig. 1. Voltages between 80 and 250 V were applied to soybean liposomes. The recordings show that the shift in Doppler frequency is proportional to the applied voltage, in accord with Eqn. 1.

Fig. 2 shows the autocorrelation function of the Fourier-transformed power spectrum from quasi-elastic light scattering of phosphatidylserine liposomes. The exponential decay clearly indicates a Lorentzian behaviour of the power spectrum. Thus it can be concluded that the spectrum originates from a homogeneous population of liposomes in the preparation. An average particle radius of $a = 1700$ Å is calculated from the linewidth. The radii of liposomes prepared from the other phospholipids were 1700 ± 600 Å. Extra care has been taken with these measurements to avoid contamination of the preparations with dust.

Before investigating the effect of four drugs on the

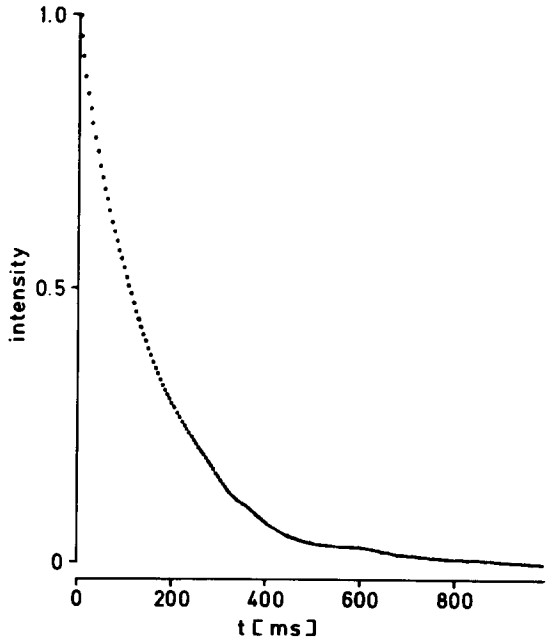

Fig. 2. Autocorrelation function from the power spectrum of phosphatidylserine liposomes in 1 mM KCl/1 mM glycine pH 7.0, at an angle of $\vartheta = 15°$.

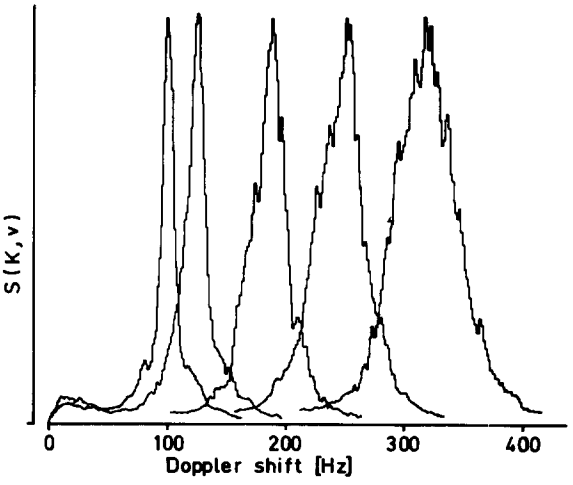

Fig. 1. Electrophoretic Doppler spectra of soybean liposomes in 1 mM KCl/1 mM glycine pH 7.0, recorded at 80, 100, 150, 200 and 250 V (from left to right).

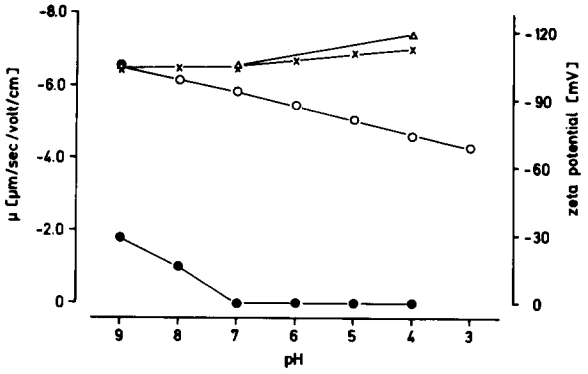

Fig. 3. Electrophoretic mobility (left-hand axis) of liposomes made from soybean lipids (circles), phosphatidylserine (crosses), cardiolipin (open triangles) and phosphatidylcholine (points) as a function of pH. The right ordinate was derived from the mobility scale according to Eqn. 3. The liposomes in a concentration of 0.33 mg/ml were suspended in 1 mM KCl/1 mM glycine.

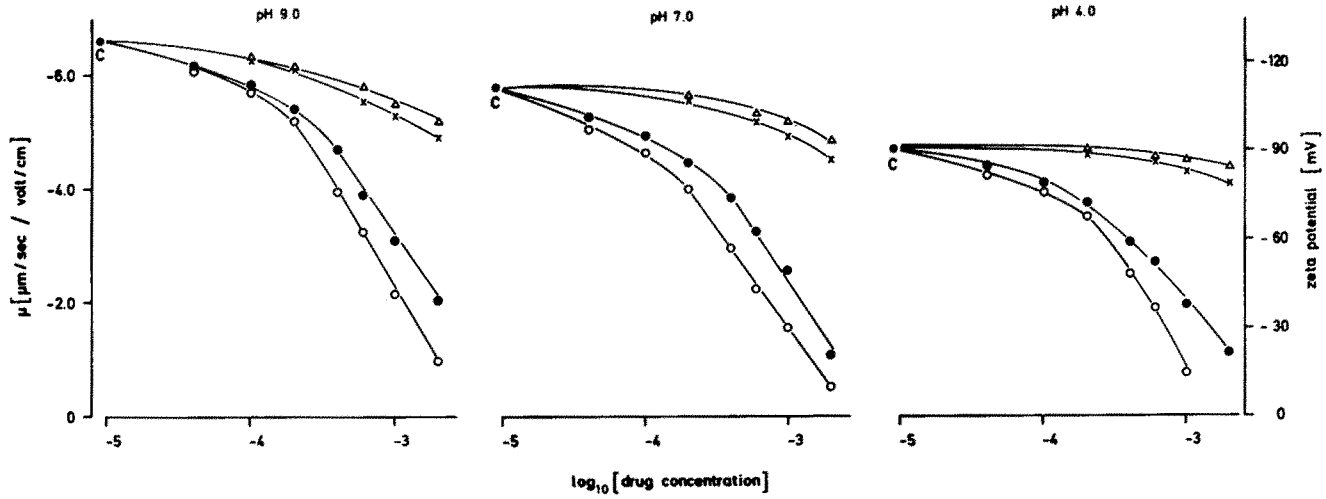

Fig. 4. Electrophoretic mobility (left ordinate) and zeta potential (right ordinate) of soybean liposomes as a function of drug concentration: ○, propranolol; ●, tetracaine; X, lidocaine; △, procaine. The liposomes in a concentration of 0.33 mg/ml were suspended in 1 mM KCl/1 mM glycine at pH 9, 7 and 4, respectively; c = control.

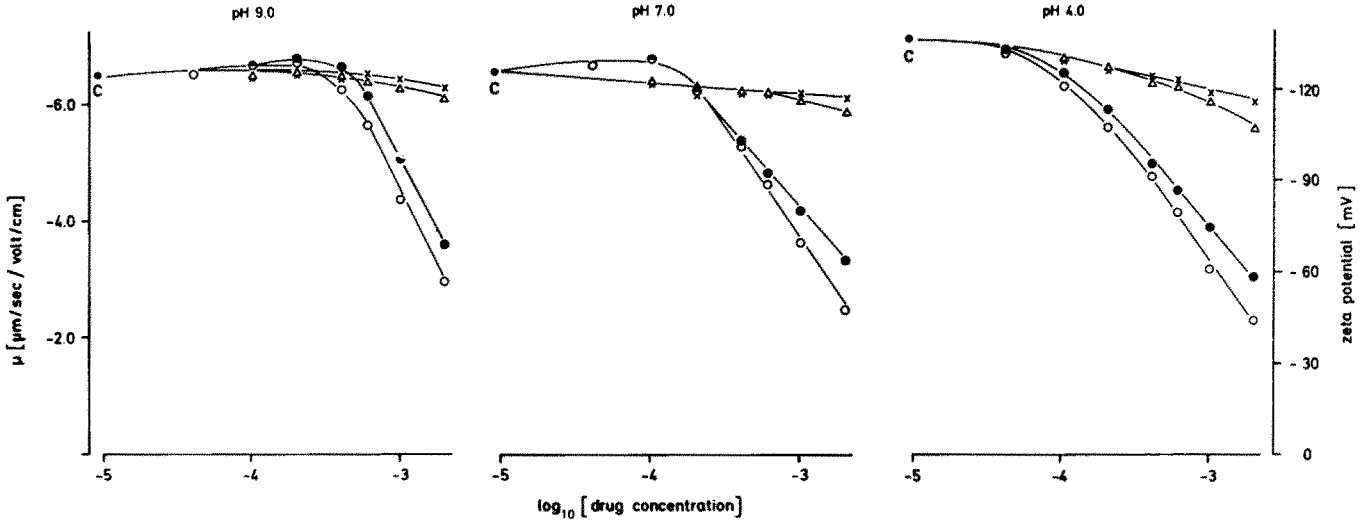

Fig. 5. Electrophoretic mobility (left ordinate) and zeta potential (right ordinate) of phosphatidylserine liposomes as a function of drug concentration: ○, propranolol; ●, tetracaine; X, lidocaine; △, procaine. The liposomes in a concentration of 0.33 mg/ml were suspended in 1 mM KCl/1 mM glycine at pH 9, 7 and 4, respectively; c = control.

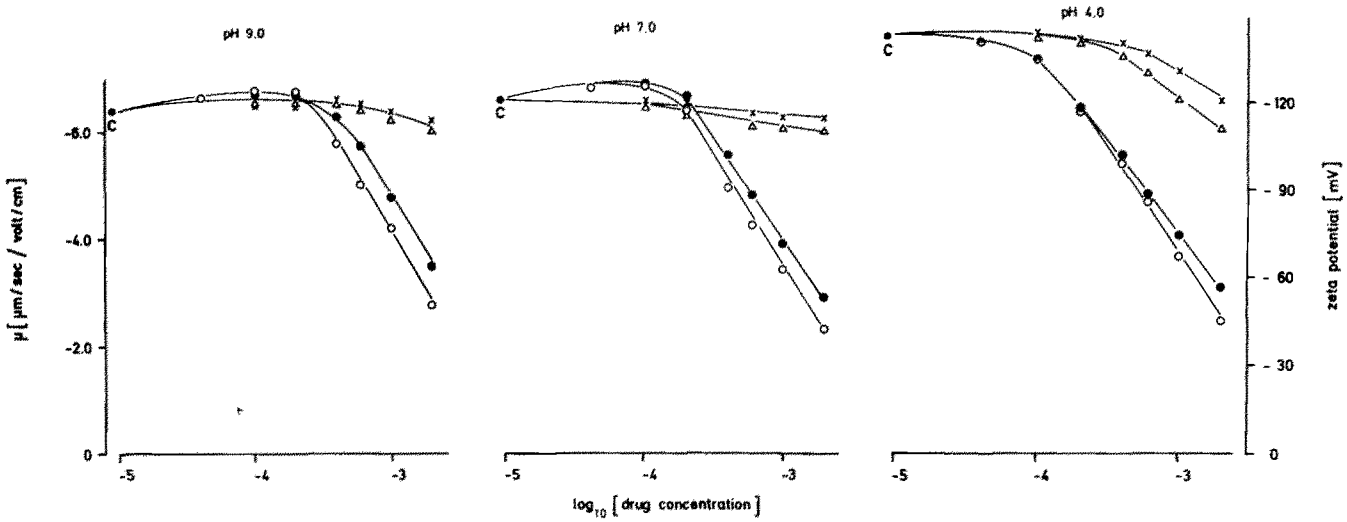

Fig. 6. Electrophoretic mobility (left ordinate) and zeta potential (right ordinate) of cardiolipin liposomes as a function of drug concentration: ○, propranolol; ●, tetracaine; X, lidocaine; △, procaine: The liposomes in a concentration of 0.33 mg/ml were suspended in 1 mM KCl/1 mM glycine at pH 9, 7 and 4, respectively; c = control.

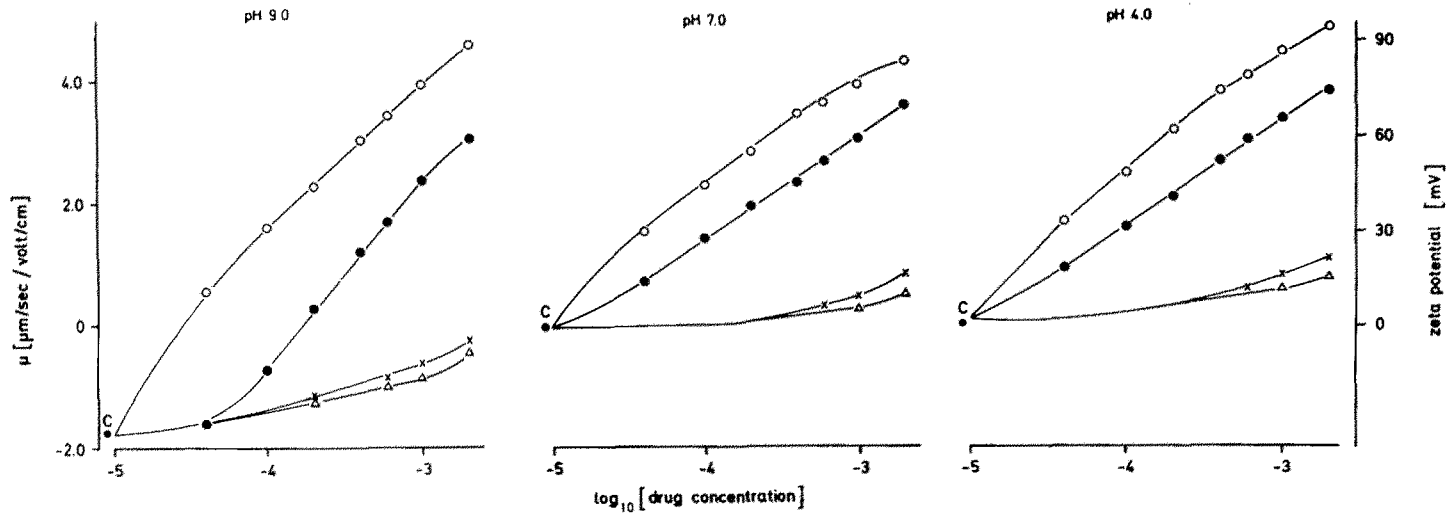

Fig. 7. Electrophoretic mobility (left ordinate) and zeta potential (right ordinate) of phosphatidylcholine liposomes as a function of drug concentration: ○, propranolol; ●, tetracaine; X, lidocaine; △, procaine. The liposomes in a concentration of 0.33 mg/ml were suspended in 1 mM KCl/1 mM glycine at pH 9, 7 and 4, respectively; c = control.

electrophoretic mobility of the liposomes at three different pH, we studied the effect of pH alone on liposome mobility.

In Fig. 3 the electrophoretic mobility and the zeta potential of liposomes made from four different phospholipids are plotted as a function of pH. The mobility of liposomes from soybean lipids decreases linearly with decreasing pH in a range between 9 and 3. The mobility of pure phosphatidylserine and cardiolipin liposomes is constant in a pH range of 9 to 7 and then increases slightly at acidic pH. There is no significant difference between these two phospholipids except at pH 4, the mobility of cardiolipin liposomes being slightly higher than that of phosphatidylserine. Liposomes made from pure phosphatidylcholine showed zero mobility at pH 7 to 4; at alkaline pH some mobility is observed.

The ζ-potential was determined from Eqn. 3 by introducing a corresponding factor for Henry's function [1].

Fig. 4 demonstrates the electrophoretic mobility of soybean liposomes as a function of drug concentration. Propranolol, tetracaine, lidocaine and procaine decrease the mobility and hence the surface potential at pH 9, 7 and 4. The following sequence of strength of activity can be derived from these results: propranolol > tetracaine > lidocaine > procaine. Effects are stronger at a pH of 9 and weaker at a pH of 4.

Similar effects are observed for liposomes made from phosphatidylserine. Here, all four drugs tested also decrease the electrophoretic mobility (Fig. 5) and the activity sequence is similar to that obtained with soybean liposomes. At all three pH values, propranolol is the most potent drug in decreasing mobility, followed by tetracaine and procaine; lidocaine is the weakest in changing mobility. At pH 9 and 7 under small concentrations of propranolol and tetracaine (40 to 200μM) the electrophoretic mobility is slightly increased before it is diminished at higher drug concentrations. This effect is not observed at pH 4, where 40 μM propranolol and tetracaine already decrease the mobility. In contrast to the soybean liposomes, the drug effects are highest at pH 4 and lowest at pH 9 for the serine liposomes.

Cardiolipin liposomes show very similar effects (Fig. 6). As with serine liposomes, mobilities increase slightly at low drug concentrations at pH 9 and 7, before they are decreased at higher concentrations. The same activity sequence is observed: propranolol > tetracaine > procaine > lidocaine. Effects are also strongest at pH 4. Effects differed from those obtained on phosphatidylserine liposomes by only about 3%.

Different results are obtained with liposomes made from pure phosphatidylcholine (Fig. 7). Between pH 7 and 4 these particles are electrically neutral and hence have no electrophoretic mobility, whereas at pH 9 an electrophoretic mobility can be recorded. At pH 7 and 4 all four drugs induced a high mobility in these liposomes while at pH 9 the mobility is first reduced to zero and then increased. Unlike the other liposomes, there is a linear relationship between effect and the log of drug concentration. The drug potency is of the same order as in soybean liposomes.

The results are summarized in Table I, where the ED_{50} values, i.e., dissociation constants, are listed. The ED_{50} values were calculated by fitting the logit function to the experimental data. The highest drug effect (zero surface potential for the negatively charged lipids) was set to 100%, except in the case of phosphatidylcholine, for which the value of the ζ-potential at 2 mM drug concentration was set to

TABLE I

ED_{50} VALUES OF PROPRANOLOL AND TETRACAINE FOR FOUR DIFFERENT LIPIDS AT pH 9, 7 AND 4

Lipid	pH	ED_{50} (mM)	
		Propranolol	Tetracaine
Phosphatidylcholine	9	0.087	0.28
	7	0.083	0.17
	4	0.09	0.15
Soybean lecithin	9	0.54	0.91
	7	0.38	0.70
	4	0.39	0.71
Phosphatidylserine	9	1.70	2.17
	7	1.26	1.84
	4	0.84	1.23
Cardiolipin	9	1.61	2.11
	7	1.11	1.55
	4	0.75	1.12

100%. Determinations could be performed only for propranolol and tetracaine, since the weak effects of procaine and lidocaine made curve fitting impossible. But as can be seen qualitatively in Figs. 4 to 7 the ED_{50} values for lidocaine and procaine range from about 10 to 30 mM.

In comparison with all the other drugs, propranolol is the most effective in changing the ζ-potential. The effects are weaker at pH 9 and stronger at pH 4 for negatively charged liposomes, while it is about the same for all three pH for electrostatically neutral or partly neutral liposomes. Generally lower effects are observed in negatively charged phospholipids, whereas the highest effect occurs in electrically neutral phosphatidylcholine.

Discussion

Introducing the measured ζ-potential values of phosphatidylserine and cardiolipin vesicles under control conditions at pH 7 into Eqn. 5 gives a surface charge density of $\sigma = 1\ e^-/949\ Å^2$ for both lipids. This value is surprisingly low. For the ideal case of a pure phospholipid with one net negative charge, a charge density of $1\ e^-/60\ Å^2$ may be expected [31, 32]. There may be two reasons for this discrepancy: (1) the ζ-potential corresponds to the potential at the plane of shear but not necessarily to the potential at the surface of the vesicle. The plane of shear, however, is located a few Ångstroms away from the solid lipid surface, where the electrostatic potential has already dropped significantly, thus leading to an underestimation of charge density. (2) The phospholipids might be contaminated by divalent ions. We have checked the effect of 1 mM EDTA on the vesicles and found no change in mobility for liposomes made from soybean lipids, phosphatidylcholine and cardiolipin. However, the mobility of phosphatidylserine liposomes was increased from $\mu = 6.5$ to $\mu = 8.0$ μm/s per V per cm, indicating a contamination of the lipid with divalent ions.

The presence of calcium was established more directly by atomic absorption spectrophotometry. All the lipids except phosphatidylcholine contained a certain amount of calcium, although the exact concentration could not be determined because the percentage of ionizable Ca^{2+}, which is strongly complexed by the phosphate group of the lipids, is not known.

It should be mentioned that other authors [10,33, 34] find charge densities of about $1\ e^-/300\ Å^2$ for phosphatidylserine, which is a better value than ours. However, the primary object of the present study was to investigate comparatively the effect of various drugs on the surface properties of different lipid vesicles, and not to demonstrate the validity of the Gouy-Chapman theory of the diffuse double layer.

The liposomes appear to be rather large. However, as analysed by Wiersema et al. [43], the mobility of liposomes in a monovalent salt solution should be essentially independent of size. In our study we were interested rather in the drug-induced mobility changes and not in absolute mobilities of particles of a definite size.

The results demonstrate that the four drugs investigated are remarkably effective in changing the ζ-potential of electrostatically charged or neutral phospholipid vesicles. For the neutral phosphatidylcholine, the uncharged drugs are incorporated into the lipid phase and become positively charged due to reprotonization of the secondary or tertiary amine group. The mobility, i.e., ζ-potential, of these vesicles increases with increasing drug concentration. When interacting with negatively charged lipid vesicles (soybean, phosphatidylserine, cardiolipin), on the other hand, the drugs decrease the negative surface potential as a result of their reprotonization. Electrically neutral lipids are more sensitive to the drugs (low ED_{50}) than are negatively charged lipids (high ED_{50}). This may be due either to the lipid-bound calcium contamination or to the negative charges, which could interfere with the reprotonization of incorporated drugs through ionic interactions at the hydrodynamic plane of shear.

It should be pointed out that the addition of the drug solution can change the pH of the medium. When interacting with the phospholipids, the drugs release protons [35], which can alter the pH. Low buffer concentrations in our experiments were especially chosen to avoid effects of the buffer itself on the ζ-potential. In a set of control experiments we observed, for the four phospholipids used, only a slight decrease in pH under the four drugs at pH 7 (from 7 to 6.8) and no pH change at 4. However, starting at pH 9, pH decreased

linearly with increasing drug concentrations (by 2 mM propranolol to 8.1, by 2 mM tetracaine to 7.7, by 2 mM lidocaine to 7.4 and by 2 mM procaine to 8.0). Consequently, some data listed under pH 9 are slightly falsified by changes in the pH. As demonstrated in Fig. 3, only the mobility of liposomes made from soybean lipids and from phosphatidylcholine vary with pH. Changing pH from 9 to 8 decreases the surface potential by 6 mV for soybean and 13 mV for phosphatidylcholine. The reported values are therefore overestimated by these potential values. The surface potential of liposomes from cardiolipin and phosphatidylserine does not vary with pH in the range of 9 to 7 and need not be corrected.

A clear difference in the strength of action of the four drugs tested may be derived from the results. Propranolol is the most effective drug in changing the surface potential of electrostatically neutral lipids, followed by tetracaine, lidocaine and procaine. A similar sequence in action intensity is also found in biological tissues. Blaustein and Goldman [36] find the following sequence for the relative effectiveness in blocking nerve conduction: tetracaine > lidocaine > procaine. Sasa et al. [37] report that propranolol is 12-times more effective than procaine in blocking excitation of lobster giant axon. Finally the results of Hille [38] indicate a sequence in anesthetic activity on frog node of Ranvier similar to that reported by Blaustein and Goldman for lobster axon.

It is surprising that the ED_{50} values of propranolol and tetracaine obtained at pH 4 in phosphatidylserine and cardiolipin liposomes are slightly smaller than those obtained at pH 9. Judging by the pK values of the drugs (9.4 [39] and 8.5 [38] respectively), one should expect higher effects in an alkaline milieu because of the higher concentration of the lipid-soluble, unprotonated free base of the anesthetics. This discrepancy may be interpreted in the following way: at acidic pH a certain concentration of the free base of the drug still exists and is incorporated into the lipid phase. Because of chemical equilibrium the protonated form of the drug in the buffer medium changes over to the unprotonated, which again can enter into the phospholipid phase until equilibrium is reached. The lipid-dissolved neutral drug partly reprotonizes at the surface of the liposome, changing the surface potential. This protonization process is stronger in an acidic milieu than in an alkaline.

Another reason for the discrepancy of ED_{50} values at pH 4 and 9 may be that negative surface charges interfere with the reprotonization process of the drug. Approaching low pH, the negatively charged phosphatidylserine and cardiolipin liposomes become more and more protonized (pK for phosphatidylserine: 4.3–5 [8]), gradually neutralizing their negative surface charge. This allows a better protonization of the drugs.

There may be still another reason for the discrepany of ED_{50}: calcium contaminants. At acidic pH Ca^{2+} is displaced by protons. This explains the mobility increase of phosphatidylserine and cardiolipin liposomes in acidic buffers (Fig. 3). Ca^{2+} and the drugs investigated are antagonists: less lipid-bound Ca^{2+} allows a better binding of the drugs. That Ca^{2+} interferes with drug binding is also seen in Figs. 5 and 6. At low drug concentrations (less than 100 μM) a small increase in mobility above the control value is observed for propranolol and tetracaine. This effect may be explained by competitive displacement of Ca^{2+} from the lipid surface by the drugs. For the zwitterionic lipid (phosphatidylcholine) and the mixture of zwitterionic and negative

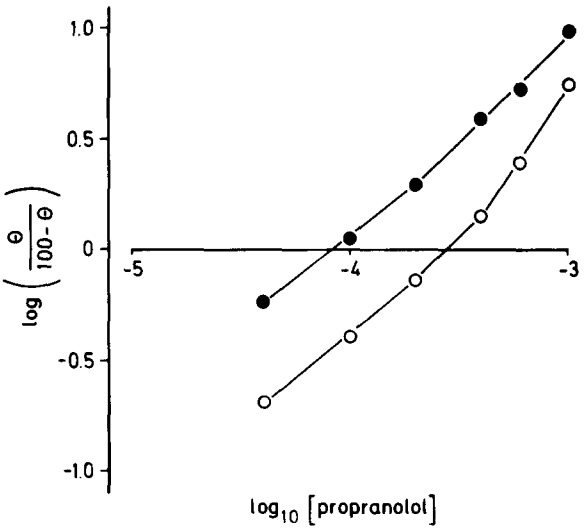

Fig. 8. Hill plot of the zeta potential changes due to propranolol in soybean liposomes (open circles) and phosphatidylcholine (filled circles) in 1 mM KCl/1 mM glycine, pH 7.

lipids (soybean) the ED_{50} values are about the same at all three pH, probably because of lower calcium contamination.

However, our data still fit the biological observations. Hille [38] shows that only the rate of onset of the anesthetic effect is raised at alkaline pH. The drug-induced decrease in sodium permeability of frog node of Ranvier is about the same at alkaline and at acidic pH under steady-state conditions.

A Hill plot of the data from soybean liposomes at pH 7 gives $n = 0.8$ for low concentrations of propranolol and $n = 1.0$ for high concentrations (Fig. 8). This result indicates a non-cooperative, independent binding of the drug to the phospholipids. A similar result holds for phosphatidylcholine. The point where the curve crosses the concentration axis gives the binding constant, which is 295 μM for soybean lipids and 84 μM for phosphatidylcholine. The value from soybean liposomes is in approximate and the one from phosphatidylcholine liposomes in very good agreement with the ED_{50}s listed in Table I.

The combination of Eqns. 5–7 is known as the Stern relation [2]. A determination of the dissociation constants based on these equations for three different propranolol concentrations on soybean liposomes yields three widely varying values. This result is explained by the fact that for simplification we introduced the measured ζ-potentials into the equations instead of ψ_o, the potential at the liposome surface. Assumptions inherent in the Gouy-Chapman relation may also play a role (for a detailed discussion see McLaughlin [4]). A much better fit was obtained from the data of neutral phosphatidylcholine liposomes. In this case $\sigma_{initial}$ is zero and Eqn. 6 reduces to

$$K_a = C_o = c \cdot e^{-(F/RT)\zeta}$$

These data may also indicate that in negatively charged liposomes the plane of shear varies more strongly with drug concentration than in electrostatically neutral phosphatidylcholine liposomes.

The half-line-width of the scattering power spectrum gives an estimated average particle size of 4600 Å diameter for soybean liposomes and 3400 Å for phosphatidylserine liposomes. No changes in linewidth were observed for drug concentrations under 2 mM. This clearly indicates that no aggregation of liposomes occurs at the drug concentrations used for our study. Therefore an effect of size change on electrophoretic mobility can be excluded.

The mean particle size can be used to determine the mean liposome density, ρ_n, and hence the average interparticle distance, s, by the equation

$$s = \left(\frac{1}{\rho_n}\right)^{1/3} \tag{11}$$

The following conditions are taken into account for this calculation: (1) the liposomes are single-walled bilayers, (2) the average molecular weight is 730, (3) the area per lipid molecule is 64 Å^2. The interparticle distance for phosphatidylserine vesicles is then $s = 2.0 \cdot 10^{-4}$ cm and for soybean liposomes $s = 3.1 \cdot 10^{-4}$ cm. In the case of multilayered liposomes greater distances are calculated. Interparticle interaction can hardly be expected at these distances.

We have shown in this study that drugs with local anesthetic activity are very effective in changing the ζ-potential of phospholipid liposomes. Phospholipids are a dominant component of biological membranes. The drug-induced change in ζ may be one explanation for the mechanism of action of local anesthetics, as has already been suggested by electrophysiologists (for reviews see Refs. 41 and 42). Propranolol, known to be a β-receptor blocking agent with strong local anesthetic activity, behaves very similarly to the other well known local anesthetics. The effects reported here are most likely related to its local anesthetic activity and not to its β-blocking ability, since the affinity of tetracaine, lidocaine and procaine to β-receptors is very low, as was measured in isolated heart tissues (Schlieper, P., unpublished results).

Acknowledgements

We would like to thank Mrs. D. Diensberg for technical assistance. The work was supported by grants from the Sonderforschungsbereich Kardiologie.

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